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Rapid Report

In Vitro Changes in Non-Facial Human Skin Following CO_2 Laser Resurfacing: A Comparison Study

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Background and Objective: **We evaluated the physical changes in human skin following CO_2 laser cutaneous resurfacing with either the Sharplan SilkTouch® handpiece or the Coherent UltraPulse® laser.**

Study Design/Materials and Methods: **Three-hundred five human tissue samples and matched controls were used. Up to five laser passes were performed per specimen. Parameters evaluated included: lateral skin shrinkage, transient temperature change, isometric tension development, elasticity change, and histologic change.**

Results: **Skin shrinkage increased in direct proportion to laser pass number. Isometric tension exponentially increased and elasticity exponentially decreased with successive laser passes. The zone of thermal denaturation for the SilkTouch® handpiece was 115 ± 15 μm, and was independent of laser pass number. The zone of thermal denaturation was patchy for the UltraPulse® laser treatments, regardless of pass number. A greater temperature increase was also measured for SilkTouch® irradiation than with the UltraPulse® laser.**

Conclusion: **The observed alterations in tissue length, tension development, and elasticity obtained with SilkTouch® or UltraPulse® treatment may contribute to the changes in clinical appearance associated with laser cutaneous resurfacing. Our findings support a role for extracellular matrix contraction in the mechanism of action for CO_2 lasers in cutaneous resurfacing.**

Key words: laser cutaneous resurfacing, tissue contraction, photoaging

INTRODUCTION

Treatment of photodamaged and aged skin has changed dramatically during the past several decades. Nonsurgical therapeutic options include tretinoin, alpha-hydroxy acids, and soft tissue augmentation. Each can reduce fine-to-medium wrinkles, giving skin a rejuvenated look [1]. Unfortunately, these therapies are transient, and deeper, more substantial wrinkles and tissue redundancy respond poorly.

Longer-lasting improvement can be obtained with rhytidectomy (i.e., face lift), chemical peel, or dermabrasion [2]. Rhytidectomy is the best

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treatment for redundant, sagging tissue and excess subcutaneous fat. However, multiple fine and coarse wrinkles, especially in the perioral and periocular regions, respond poorly to rhytidectomy, and uneven pigmentation is rarely improved. Chemical peel and/or dermabrasion may provide excellent results in patients with extensive coarse wrinkling and blotchy pigmentation [2,3]. However, both chemical peel and dermabrasion may produce prominent hypopigmentation, persistent erythema, irregular hyperpigmentation, or hypertrophic scarring [2,3]. Furthermore, phenol peeling has been associated with cardiac arrhythmias [4], and dermabrasion exposes operating room personnel to blood-borne pathogens. For these reasons, physicians have sought other modalities for long-lasting amelioration of aging facial skin and uneven pigmentation.

The carbon dioxide (CO_2) laser has been extensively utilized in dermatology and plastic surgery [5,6]. It has been used as a "light scalpel" for incisional or excisional procedures, and as a volume ablation tool for removal of epidermis and superficial dermis. Volume ablation and dermal shrinkage are thought to be the basis of the clinical improvement seen with the new cutaneous resurfacing lasers [6]. These lasers produce a more uniform injury compared to chemical peel or dermabrasion, thereby minimizing interoperator variability. Cutaneous shrinkage is thought to be a separate mechanism of action induced by these lasers that increases their efficacy compared to chemical peel or dermabrasion. Although no studies have been reported, it is likely that the plume generated by these lasers contains biologically active material and has a similar exposure hazard to standard CO_2 lasers.

Several studies have documented the encouraging clinical results obtained with laser cutaneous resurfacing [7–17]. However, few previous papers have examined the proposed mechanisms of actions of these lasers. We therefore studied the acute physical and histologic cutaneous changes induced by resurfacing lasers. The parameters we evaluated included lateral skin shrinkage, transient temperature change, isometric tension development, elasticity change, and histologic change. This study also compared the performances of two different commonly used cutaneous resurfacing lasers: the Sharplan SilkTouch® and the Coherent UltraPulse® lasers. The UltraPulse® laser is a pulsed laser with approximately a 1 msec pulse duration. This is approximately the same as the thermal relaxation time of the skin [18]. The SilkTouch® handpiece moves the continuous beam laser spot in a spiral during the 0.2 second exposure time. The skin "dwell" time to the spiraling beam is approximately 1 msec. Both lasers operate at 10.6 μm and have a penetration depth of 20–40 μm, depending upon tissue water, with negligible scatter.

MATERIALS AND METHODS

Tissue

Excess human skin with subcutaneous fat excised that was harvested during reduction mammoplasties was used within 72 hours for all experiments. The experimental protocol was approved by the Vanderbilt University Institutional Review Board. A plastic template was used to cut 10 × 20 mm sections. These were bisected (5 × 20 mm), providing matched controls for all experiments. Specimens and controls were stored overnight at 4°C in phosphate buffered saline. Three-hundred five tissue samples and matched controls were used for these experiments. At least 10 tissue samples were used for each data point in this study.

Lasers

A Sharplan SilkTouch® (Allendale, NJ) 125 mm hand piece with a 3.4 mm spiral-scanned pattern was connected to a Sharplan 1060 CO_2 laser set at 8W with a 200 msec repeated pulse at 1 Hz for a total of 1.6 J. The handpiece was set to the "+" spot size. Although the "spot size" was stated by the manufacturer to be 3.7–4.0 mm, we measured a 3.4 mm spiral. The Coherent UltraPulse® 5000C (Palo Alto, CA) laser settings were 2 W with a 4 Hz repeated pulse for a total of 500 mJ. A 2.0 mm measured round spot size was produced with a collimated hand piece for which the manufacturer claimed a 3.0 mm spot size. Therefore, we would measure fluences of 17.6 and 15.9 J/cm2 for the SilkTouch® and the UltraPulse®, respectively. The manufacturers would claim fluences of 13.7 and 7.1 J/cm2 for the SilkTouch® and UltraPulse® lasers, respectively.

Operating Parameters

Each set of experiments was performed by the same operator to reduce interoperator variability. Tissue within a 5 × 10 mm area centrally placed within the 5 × 20 mm strip was completely irradiated with minimally overlapping pulses during each laser pass. Each tissue specimen received from one to five laser passes. Fol-

lowing each pass, char was removed and tissue rehydrated using a cotton-tipped applicator moistened with normal saline. All samples were blotted dry before the next pass.

Tissue Shrinkage

Tissue shrinkage was measured following irradiation by evaluating the distance between two centrally-placed India ink tattoos 12.7 mm apart along the length of the tissue. The tissue was photographed before irradiation and subsequent to each laser pass and char removal. To measure shrinkage for specimens irradiated with the Silk-Touch® laser, digitalized photographs were produced with an Ikegami MKC-301A video camera (Tokyo, Japan) mounted to the sideport of an OPHI surgical microscope with a 400 mm focal length lens (Zeiss, Germany). The camera was interfaced with the built-in video port of a Mac 840 AV microcomputer (Cupertino, CA). Distances were measured with Adobe Photoshop 2.0 (Mountainview, CA). An Olympus OM-1 35 mm camera with a 3.5, f=50 mm macrolens and a ring flash was used to photograph tissues irradiated with the UltraPulse® laser. A fixed camera focal length of 9″ was used, giving a magnification of 0.5. Distances were measured in millimeters by projecting the 35 mm slides on a Telex® Caramate 4000 projector, magnifying the slides by a factor of 6.4. The resolution was better than 0.05 mm for all measurements.

Temperature Change

Changes in tissue temperature during irradiation were monitored with a Waite TCM310 digital thermometer (Culver City, CA) and a type T thermocouple. The type T thermocouple was more appropriate than an infrared thermographic device, since the thermocouple could measure dermal temperature. The approximate 1 msec thermal response time of the temperature probe corresponds closely to the thermal relaxation time of the skin [18]. The probe was placed nearly parallel to the epidermis so that the tip rested under the area to be irradiated. The depth of the probe was visually judged to be 400 to 500 μm below the surface. The probe was placed by the same operator for each specimen. The initial and peak temperatures were recorded for each pass.

Tension Measurement

Tension developed during laser treatment was measured with a device of our design (Fig. 1). A Newport 271 labjack (Irvine, CA) with adjustable platform height was positioned on the console's floor. A Mettler PJ3600 (Hightstown, NJ) electronic balance was used to measure tension. Surgical nylon thread was tied to each end of a Fisher 05-832 acetal tubing clamp (Pittsburgh, PA). The tread was looped onto a hook located on the undersurface of the balance. One end of the specimen was bound to the clamp attached to the scale. The other end was secured with an identical clamp fixed to the adjustable platform. The tissue tension was then adjusted to 20 g. Following equilibration of tension, the first pass was performed and peak tension was monitored on the balance. After char removal, rehydration, and equilibration, resting tension was recorded before proceeding with the next pass. This same procedure was followed for subsequent passes.

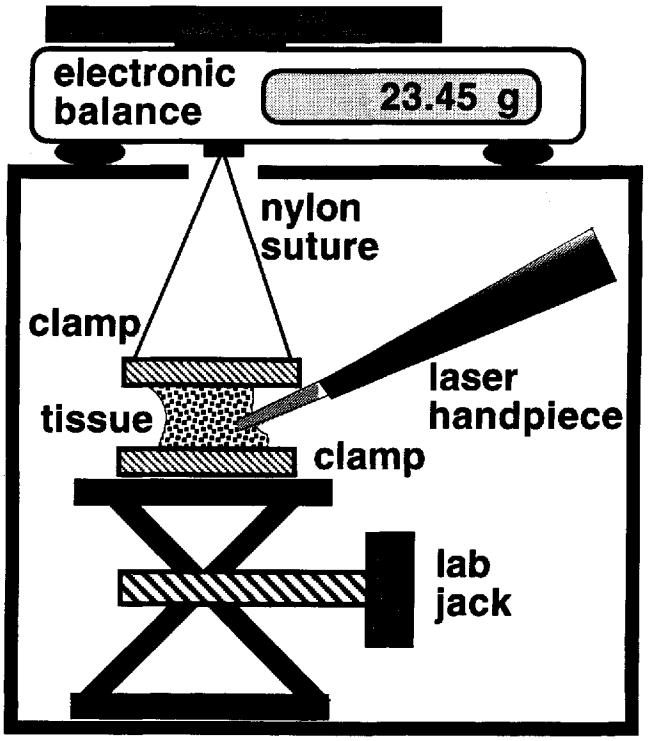

Fig. 1. Schematic of in situ tensiometer.

Elasticity Measurement

Elasticity was evaluated for both irradiated and matched control samples. The tissue was centered and mounted lengthwise into the upper and lower clamps of an Instron tensiometer (model 1130, Canton, MA). No initial tension was placed on the tissue. Tensile force was measured as a function of the distance the tissue was stretched. This determined the elasticity, or the spring con-

stant, for the tissue sample. Elasticity was evaluated for only the first 2–3 mm of tissue stretch, because physical deformation of the tissue occurred beyond this point. To control for differences between different skin samples data was expressed as the fraction of elasticity. This was calculated by dividing the mean elasticity of an irradiated specimen by the mean elasticity of its matched control.

Histologic Change

Tissue specimens were fixed in 10% formalin, embedded in paraffin, sectioned, and stained with either hematoxylin and eosin or Gomori's trichrome for light microscopic examination. Analysis was performed with an Olympus Vanox AH-2 light microscope (Lake Success, NY). Morphometric measurements were quantified using Southern Micro Instruments planar morphometry software (Atlanta, GA).

RESULTS

Tissue Shrinkage

Tissue contracted when irradiated with either laser (Fig. 2). The amount of shrinkage was linearly related to the number of passes (R_2 = 0.983 and 0.958 for the SilkTouch® and UltraPulse® lasers, respectively). The SilkTouch® produced a 6% size reduction per pass ($P < 0.001$). The UltraPulse® produced a 5% size reduction per pass ($P < 0.001$). There was no statistical difference between the slopes ($P > 0.05$). Statistically, straight lines provided the best fits for the two data sets. Although a fraction of the tissue shrinkage disappeared when the tissue was rehydrated, a significant amount of shrinkage persisted (Fig. 3). The tissue rehydration was accomplished by rubbing the irradiated skin with a cotton-tipped applicator moistened with normal saline in a manner similar to clinical treatments. No attempt was made to regulate the amount of rehydration.

Temperature Change

The tissue temperature increased during treatment with either laser. The average temperature increase above ambient temperature (20–25°C) was 19.5°C ± 1.9°C with the SilkTouch® and 15.4°C ± 1.0°C with the UltraPulse® laser. The temperature increase was significantly greater with the SilkTouch® laser ($P < 0.001$). The magnitude of temperature increase was independent of pass number for either laser.

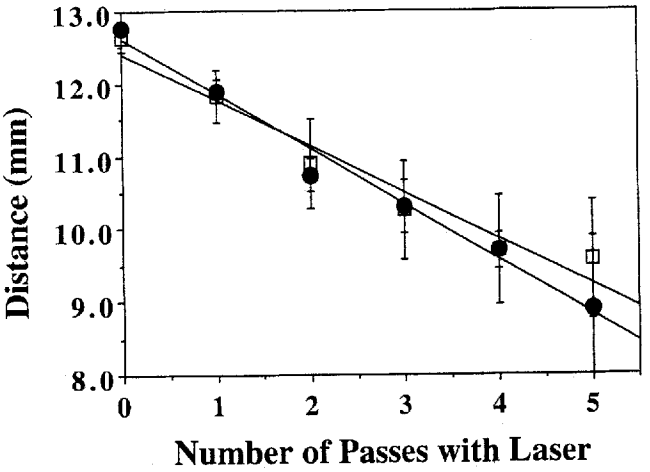

Fig. 2. Shrinkage as a function of irradiation. Mean distance (mm) between ink tattoos ± S.E.M. is plotted as a function of number of laser passes. The straight line fit is from a linear regression. (•) represents SilkTouch® irradiation and (□) represents UltraPulse® irradiation.

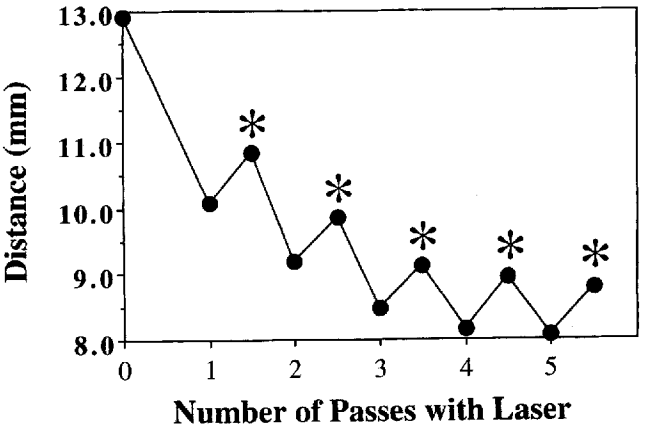

Fig. 3. Hydration effects on shrinkage as a function of irradiation with the SilkTouch® laser on a single representative tissue sample. Distance (mm) between ink tattoos is plotted as a function of the number of laser passes. Points corresponding to numbered passes represent tissue immediately after laser treatment. Points labeled with an asterisk represent tissue after char removal and rehydration.

Tensiometric Measurement

Tissue irradiation increased the tissue tension, which declined to a significantly lower resting tension after rehydration ($P < 0.05$) (Fig. 4). Both peak and resting tensions plateaued after an initial increase with laser treatment. The resting tension plateau was attained after the second pass with the SilkTouch® and after the third pass with the Ultrapulse® laser. There was no statistical difference between the two plateau levels for the two lasers ($P > 0.05$ for both).

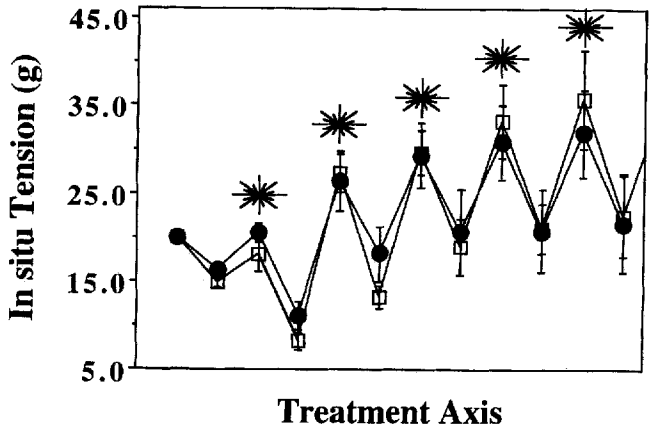

Fig. 4. Tension changes during laser treatment. Mean tissue tension ± S.E.M. (in g) is plotted during the course of tension experiments. Sunburst symbols above the peaks indicate laser treatment. Nadirs following peaks represent equilibration of tension after char removal and tissue rehydration. A tensile force of 20 g was initially applied and allowed to equilibrate before the first pass. SilkTouch® irradiation is depicted by filled circles (●) and Ultrapulse® irradiation is depicted by open squares (□).

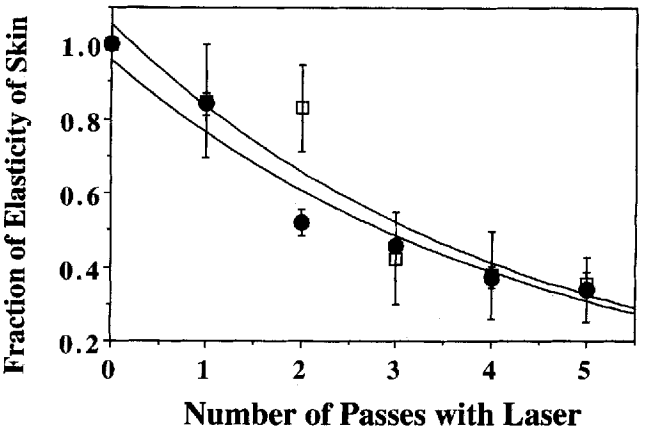

Fig. 5. Changes in elasticity following irradiation. Mean fraction of elasticity ± S.E.M. is plotted as a function of number of laser passes. SilkTouch® irradiation is represented by filled circles (●) and UltraPulse® irradiation is represented by open squares (□).

Elasticity Measurement

The elasticity of laser-treated specimens decreased as the number of laser passes increased (Fig. 5). The fraction of elasticity decreased exponentially with increased passes using both the SilkTouch® and the UltraPulse® lasers (R^2 = 0.948 and 0.896, respectively). There was no statistical difference between the slopes of the curves ($P > 0.05$).

Histologic Tissue Changes

Microscopic examination of laser treated or control skin samples was done on slides stained with hematoxylin and eosin (routine histology) or Gomori's trichrome (collagen stain). The amount of tissue ablation observed was similar for both lasers (Fig. 6). The first pass of the laser appeared to ablate the epidermis and some of the papillary dermis. Further passes resulted in further ablation. Tissue morphometry was done to quantify observed histologic changes. A band of collagen denaturation, observed by dermal tincture change, appeared lighter green on specimens irradiated with the SilkTouch® laser (Fig. 6B,D,F as noted by the arrowheads). The depth of collagen denaturation remained relatively constant at 115 ± 15 μm following each pass with the SilkTouch® laser (Fig. 7). The tissue sample thickness initially increased with the first two passes, but decreased with further passes (Fig. 7). A marked decrease was seen after five passes. Tissue samples irradiated with the UltraPulse® laser showed ablation, but in contrast to the SilkTouch® laser, only patchy areas of collagen denaturation were seen, which were not quantifiable by morphometry (Fig. 6C,E).

DISCUSSION

The search for a more effective modality to treat facial wrinkles and photodamaged skin led to the development of laser cutaneous resurfacing. We studied the physical changes in human skin which may contribute to the clinically observed cutaneous changes. Our study evaluated lateral skin shrinkage, transient temperature change, isometric tension development, elasticity change, and histologic change. We also sought to characterize any differences between two of the commonly used laser modalities, the SilkTouch® and the UltraPulse® lasers.

The tissue model used in this study has some notable differences from the usual clinical in vivo usage of CO_2 resurfacing lasers. In vitro skin samples do not have tissue cooling from blood flow, perspiration, or other factors. These skin samples also were from breast skin which would not be expected to have the same degree of actinic damage as that seen on the face. In addition, the presence of the platysma muscle below the mandibular line and beneath the breast skin was ignored in this study. In spite of these limitations, we believe this model is justified and useful for

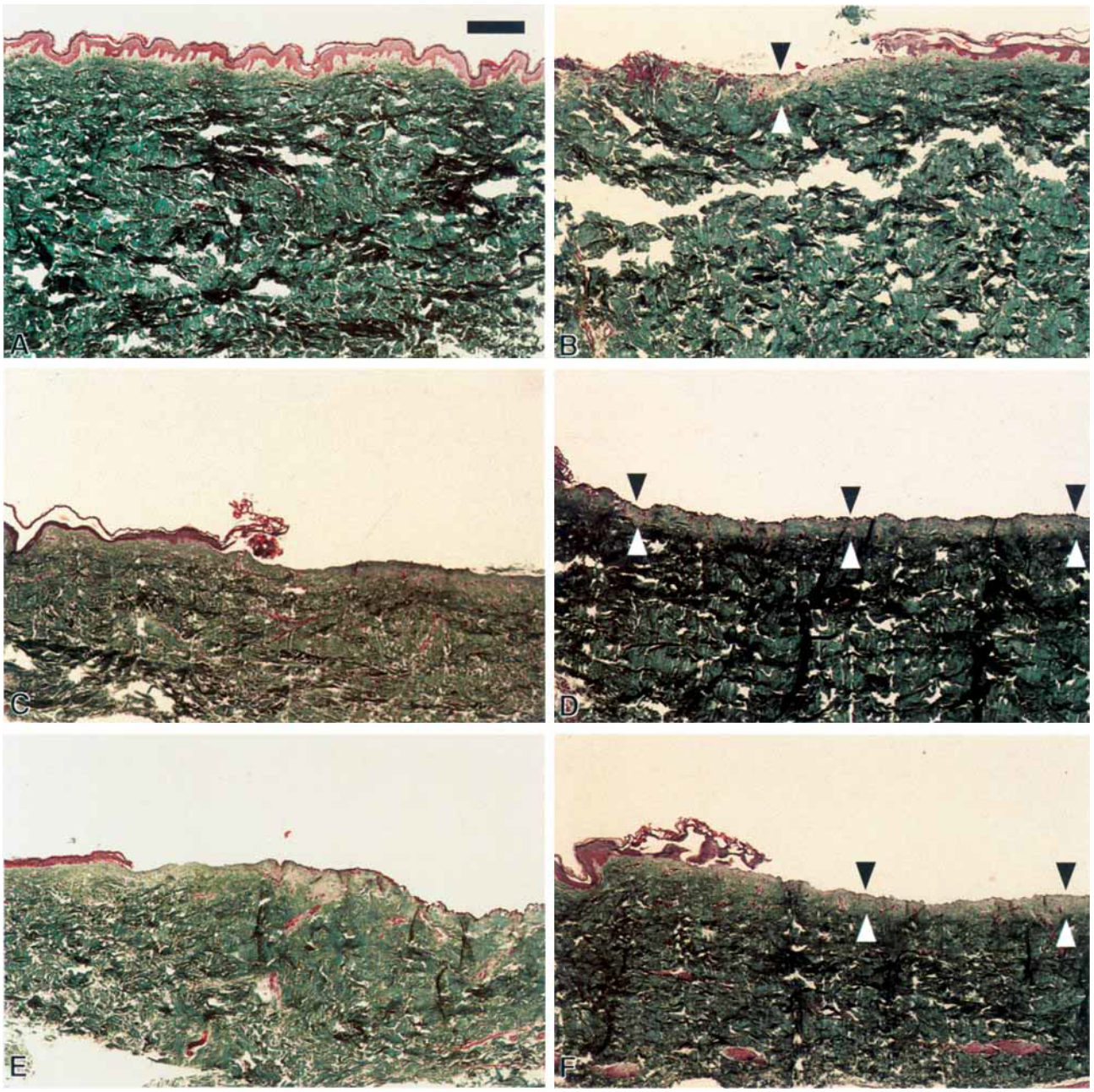

Fig. 6. Histology of laser treated and control tissue specimens. Gomori trichrome stain, all photographs taken at 10×. Size bar = 300 micrometers. Arrowheads denote the zones of collagen denaturation seen with the SilkTouch laser treatments. **A:** Control tissue section, matched control to Figure 6B. **B:** SilkTouch® laser treatment, one pass. **C:** UltraPulse® laser treatment, one pass. **D:** SilkTouch® laser treatment, three passes. **E:** Ultrapulse® laser treatment, five passes. **F:** SilkTouch® laser treatment, five passes.

studying tissue shrinkage from thermal effects on collagen.

Our results indicate that laser cutaneous resurfacing produced acute tissue contraction. A linear relationship existed between shrinkage and the number of laser passes. There was no statistical difference between the amount of shrinkage created by the two lasers. A fraction of the shrinkage disappeared with tissue rehydration. However, the remaining shrinkage implied that a lasting effect may be imparted by these lasers. Shrinkage may have progressively increased be-

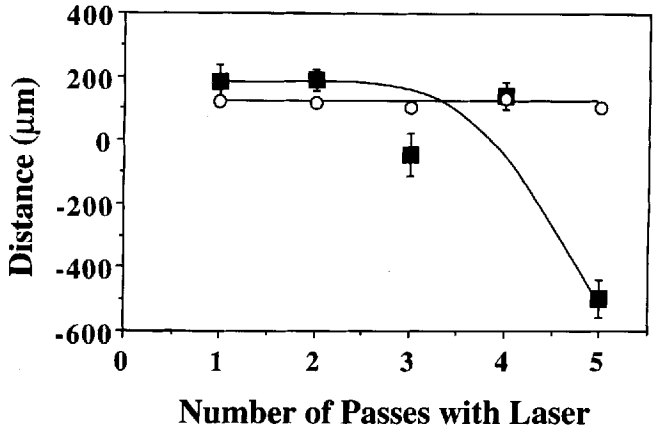

Fig. 7. Collagen denaturation and tissue thickness changes following irradiation. Mean depth of denaturation is plotted as a function of number of laser passes for the SilkTouch laser (open circles). The S.E.M. is approximately the symbol size, and is not shown. The solid squares represent changes in the tissue thickness ± S.E.M. as a function of the number of laser passes done with the SilkTouch® laser. Lines are drawn to aid in demonstrating the observed trends with the laser treatments.

cause each pass removed a relatively constant portion of tissue. With less tissue to resist the contractile forces of the denatured collagen, the amount of shrinkage increased. Because the amount of tissue removed and the depth of collagen denaturation remained relatively constant with each pass, shrinkage proceeded linearly.

The CO_2 laser produced rapid, localized heat production in the tissue. Our data indicated the temperature change was significantly greater with the SilkTouch® laser. Although the difference between the two lasers was statistically significant, the clinical importance of this result is less certain. It is of note that our temperature probe had approximately a 1 msec response time. This correlates well with the thermal relaxation time of tissue [18]. However, the UltraPulse laser may have created a temperature spike that was too fast to be completely measured by our temperature probe. Again, the clinical importance of such a rapid temperature transient is unknown. In vivo experiments therefore need to be conducted to determine whether these observed very short-lived temperature differences have an impact on wound healing and if the ultimate clinical outcomes differ for the two lasers.

Tissue tension increased with laser irradiation, followed by equilibration to a significantly lower resting level. Peak and resting tensions attained plateau levels with both lasers. However, the resting tension plateau was reached after the second pass with the SilkTouch® and the third pass with the UltraPulse®. This difference was most likely the result of the SilkTouch® laser's known ability to ablate more tissue during a single laser pass [7]. Collagen denaturation probably provided the net contractile forces needed to increase the peak and resting tensions to their constant plateau levels. After attainment of plateau levels, the subsequent rises and falls in tension were probably the result of changes in tissue hydration. Because the tension applied to the tissue was relatively small and the depth of collagen denaturation remained similar throughout laser treatment, more laser passes did not appreciably affect the plateau levels. Although our experimental evidence is indirect, our data suggests thermal effects of the laser led to the collagen denaturation and tissue contraction observed. While it has been suggested that laser cutaneous resurfacing only removes the solar elastotic tissue and permits the remaining skin to contract, our data disputes that model. We measure significant contraction in skin without appreciable solar elastotic tissue.

The fraction of elasticity decreased exponentially with increasing number of laser passes. This laser-induced decrease in elasticity was probably due in large part to denaturing and ablating tissue collagen and elastin. Because the relative thickness of collagen denaturation remained constant, tissue ablation was most likely responsible for the exponential decrease in elasticity. Another factor in the exponential decrease in the fraction of elasticity may be due to the type of tissue being vaporized. The first pass of each laser removed only the epidermis, and left thermal damage in the papillary dermis. The second pass removed the papillary dermis and left thermal damage in the reticular dermis. Subsequent passes removed reticular dermis, leaving thermal damage in the remaining reticular dermis. Reticular dermal collagen is denser and more highly organized and cross-linked than papillary dermal collagen. Therefore, thermal damage to the different collagen layers may have contributed to the observed exponential decrease in elasticity.

Light microscopy confirmed that irradiation with resurfacing CO_2 lasers produced collagen denaturation and ablation. Minor differences were observed with the two lasers. While the SilkTouch® laser produced a well defined zone of collagen denaturation, the UltraPulse® laser produced a patchy, poorly defined area of collagen

denaturation. These differences may have been, in part, due to the observed lower increase in tissue temperature with the UltraPulse® laser as compared to the SilkTouch® laser. Our histologic findings are similar to that reported by Chernoff et al. [14] for the SilkTouch® laser, as they observed a thermal necrosis depth less than 150 μm. Methods of measurement and S.E.M. were not reported. Our results also compare favorably with that seen by Kuvar et al. [19] for the UltraPulse 5000 where a pulse energy of 450 mJ was used at 4 W with the 3 mm collimated Truespot handpiece, and from one to three passes were done with the laser. We found similar patchy coagulation necrosis to that seen on their histology slides, although we were unable to duplicate their reported increase in depth of coagulation necrosis produced with increasing passes of the laser. The number of specimens, number of measurements, and S.E.M. were not reported.

It is also possible that the full extent of the thermal damage was not visible in these acute studies. Additional thermal damage would probably be evident if the tissue survived for 48 to 72 hr after the treatment. Using a different (superpulse) CO_2 laser system (the XJ-150, direct current CO_2 laser, Sharplan Lasers Inc., Allendale, NJ), Cotton et al. [20] observed 90–190 μm of dermal coagulative change with one pass of the laser at 400 mJ, 10 w, 0.33 second interval and 100–260 μm of dermal coagulative change with two passes at the same settings in specimens collected 24 hours after treatment. Tissue was not obtained immediately after wounding in this study, so the acute effects of the laser can not be directly compared with our study. The wounds seen with their laser system were similar to that observed with a medium depth chemical peel.

The observed shrinkage of the laser-treated tissue also appeared to result in reduction of the depth of the wound bed histologically (tissue thickness) compared to what might be expected with simple ablation of tissue (Fig. 7). This effect on wound depth may contribute to enhanced wound healing by decreasing the amount of granulation tissue required for healing. The clinical significance of these observations are unknown and require further investigation.

CONCLUSIONS

CO_2 laser cutaneous resurfacing produced epidermal ablation followed by collagen denaturation and ablation, as expected. Tension development and elasticity displayed the greatest amount of change during the first two to three laser passes. Shrinkage progressively increased with more laser passes. The SilkTouch® and the UltraPulse® lasers rendered similar performances for most parameters evaluated. However, the transient temperature increases during laser irradiation and the observed zones of collagen denaturation were significantly greater for the SilkTouch® laser. Further in vivo studies including wound healing studies that examine extracellular matrix repair must be performed to evaluate the significance of these findings.

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